Micro-Organisms and Soil Structure Affect Yellow-Poplar Growth

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Old-field hardwood plantings in the Central States Region have generally resulted in poor survival and growth. On such sites soil structure, organic matter content, fertility, and other factors related to tree growth differ greatly from those of the original, complex forest soil. The effects of the various old-field soil factors on tree growth have not been explained. Nor have the effects of volunteer vegetation or planted trees on the site been evaluated in terms of tree growth. Once the basic growth responses of hardwoods on old-field sites are better understood, efforts to re-establish hardwoods can be undertaken with more certainty. Therefore in 1959 a series of studies with container-grown yellow-poplar (*Liriodendron tulipifera* L.) seedlings was begun to determine the effects of micro-organisms and soil structure on tree growth.

Methods

Soil samples for growing seedlings were taken from various parts of an old field and an adjacent forest on a broad ridge in Orange County, Indiana. In 1937 part of the field was planted with shortleaf pine (*Pinus echinata* Mill.), part was planted with black locust (*Robinia pseudoacacia* L.), and the remainder was left unplanted. In 1960 stocking for the pine and locust plantation sample sites was 140 and 70 square feet of basal area per acre. The edges of the unplanted area have been invaded by trees while the center has remained in weeds and grasses. Soil samples were taken where the old field was fully stocked (88 square feet) with volunteer hardwoods and where the field was about half stocked (41 square feet) with volunteers. Sassafras (*Sassafras albidum* (Nutt.) Nees), persimmon (*Diospyros virginiana* L.), white ash (*Fraxinus americana* L.), and sugar maple (*Acer saccharum* Marsh.) were the most common volunteers and the trees ranged up to 25 years old. All parts of the old field had been eroded before planting, especially the area planted with black locust. The forest site is adjacent to the old field and is occupied by second-growth upland hardwoods. The soils are unglaciated silt-loams, acid in reaction, and are in the Wellston, Zanesville, Tilsit series. Undoubtedly the sample areas differed somewhat in soil and other site characteristics but the different vegetational covers provided a unique opportunity to relate gross site changes to cover types.

A method described previously[1] was devised to move soil samples to the laboratory with soil structure intact. Briefly, the open end of a number 10 tin can is firmly pressed against the sampling surface while the litter is cut with a knife or trowel. The can is driven into the ground nearly flush with the soil surface by means of a wooden block and a sledge hammer. Air escapes through holes punched in the end of the can. The can and its contents are carefully removed with a shovel and the excess soil shaved from the bottom.

When the sample is ready for treatment or planting, the top of the can is removed with a can opener. The sample is then an 0.8-

[1]Clark, F. Bryan. Pot culture — an aid to site evaluation. Ind. Acad. Sci. Proc. 1960, 70: 234-237, illus. 1961.

gallon soil core enclosed in a tin sleeve open on both ends. The bottoms of the containers are covered with heavy plastic held in place with rubber bands. To prevent water-logging the plastic bottoms are punctured. The best time to take samples is when the soil moisture is near field capacity. Roots, rocks, and tight soil cause cans to collapse, hence some loss is unavoidable.

To find out what effect micro-organisms have on yellow-poplar seedling growth, containers of soil were sterilized by gassing with methyl bromide. Some containers of sterilized soil were "inoculated" with forest soil by placing ⅜-inch-diameter plugs of unsterilized soil into holes punched in the sterilized soil. In other containers macerated yellow-poplar roots presumed to contain endotrophic mycorrhizal fungi (a fungus that naturally infects yellow-poplar) were placed in small holes made in the sterilized soil. These roots, obtained from forest-grown yellow-poplar seedlings, were prepared by washing in distilled water, bathing in a 0.5-percent water solution of sodium hypochlorite, and rewashing in distilled water to minimize contamination. The cleaned roots were cut up and pulped in a mortar.

Soil structure was another study variable. In a preliminary experiment macro-structure was destroyed by sieving soil samples through a 2-millimeter screen. Soil was air-dried in order to pass it through this small screen. In later studies, samples were sieved moist through a 9.52-millimeter screen so that micro-organisms would not be seriously affected.

The soil in some of the containers was fertilized to find out if endotrophic mycorrhizae influence nutrient absorption in yellow-poplar seedlings. Various studies have shown that nutrient level directly affects the growth of aseptic seedlings normally infected by ectotrophic mycorrhizal fungi.

Ammonium nitrate was added to sterilized and unsterilized containers at the rate of 400 pounds of actual nitrogen per acre. Also, a 6-24-12 commercial fertilizer was used at the rate of 600 pounds per acre. At this rate 36 pounds of nitrogen, 144 pounds of phosphate, and 72 pounds of potash were added per acre. The fertilizers were finely ground, put in a water solution, and divided into five equal parts. One part was added to the soil every 2 weeks.

Yellow-poplar seeds were germinated in trays and planted in the containers when the radicals were about ¼ inch long. The first experiment was conducted outside in full sunlight. In three other experiments plants were grown in the laboratory under artificial light on a 14-hour "day." Plants grown outside were given supplemental tap water when rainfall was insufficient; plants grown inside were watered with distilled water. The seedlings were grown for 12 weeks and then removed intact from the containers by soaking and washing. Seedlings were measured and weighed fresh. Roots were preserved and sent to Beltsville, Maryland, for mycorrhizal examination.[2]

Results

In just 12 weeks, growth differences due to site quality were apparent. Seedlings grown in containers of natural forest soil weighed four times as much as seedlings grown in old-field soil that had supported weeds and grass. Results in all four trials were very uniform and three replications of each treatment were sufficient to demonstrate statistical differences. Practically all differences among treatments and sites were statistically significant at 1 percent.

Considering only the old-field samples, seedlings in soil from the weed-and-grass cover grew slowest and seedlings in containers of soil from pine and black locust plantations grew fastest (table 1). In fact, seedlings from plantation soils grew nearly as fast as seedlings grown in forest soil (fig. 1). This supports observations that old-field sites are improving under planted pine and locust. Seedlings in containers of soil from that part of the old field partially stocked with volunteer hardwoods were about the same size as those in containers from the unstocked part. However, seedlings in containers from the old-field site fully stocked with volunteers showed by their greater height that site improvement has taken place.

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[2]Examinations for endotrophic mycorrhiza were made by Edward Hacskaylo, Plant Physiologist, Forest Service, U.S. Department of Agriculture.

FIGURE 1.—Yellow-poplar seedlings 12 weeks old show that plantations improved old-field sites.

TABLE 1.—Seedling size after 12 weeks in soil under different old-field covers

Cover	Average seedling: Top Height	Average seedling: Total fresh weight
	Inches	Grams
Weeds and grass	5.9	2.2
Half-stocked hardwood stand	6.1	2.8
Fully stocked hardwood stand	7.8	5.3
Shortleaf pine plantation	7.0	7.7
Black locust plantation	7.7	9.4

Soil Structure Important to Growth

The effects of sieving on seedling growth was striking and the importance of soil structure was clearly demonstrated. Much of the difference in seedling growth in pots of soil from the various sites studied can be attributed to soil structure. Seedlings grown in forest soil sieved through a 2-millimeter mesh screen weighed less than a tenth as much as seedlings grown in undisturbed forest soil. And seedlings grown in forest soil sieved through a 9.52-millimeter mesh screen weighed about a sixth as much as seedlings grown in undisturbed forest soil (fig. 2). This difference was significant at 1 percent. Sieving pine-plantation soil also reduced seedling growth. However, seedlings grown in unsieved old-field soil were only slightly larger than seedlings grown in sieved old-field soil.

A few weeks after watering was begun, the laboratory containers with sieved soils showed signs of poor drainage and aeration. The containers are only 7 inches tall and drainage of gravitational water is probably not as complete as drainage under field conditions. Consequently, the effect of sieving may be exaggerated under laboratory conditions. But the fact remains that destroying macro-soil-structure drastically retards yellow-poplar seedling growth. The sieved soil did not become tight but remained friable

FIGURE 2. — Seedlings grown in undisturbed forest soil were six times as large as seedlings grown in forest soil sieved through a large mesh screen.

throughout the 12 weeks and seedlings were very easy to remove from the containers. In contrast, seedlings from the unsieved soil, especially in the old-field containers, were difficult to remove from the containers with roots intact.

Micro-Organisms Essential for Good Yellow-Poplar Growth

Seedlings in sterilized soil were consistently smaller than seedlings in unsterilized soil. Reduced growth caused by sterilization was outstanding in forest soil: seedlings in unsterilized containers were four times taller and weighed five times as much as seedlings grown in sterilized containers. These differences were significant at the 1 percent level. Seedlings grown in sterilized containers were also chlorotic. Seedlings from containers of soil that was sieved and sterilized were smaller than seedlings from any other treatment.

Sterilized soil inoculated with forest soil or macerated yellow-poplar roots demonstrated emphatically that micro-organisms play an important role in yellow-poplar growth. Both sources of inoculum caused much faster seedling growth. Seedlings in inoculated soil were as large as seedlings grown in undisturbed forest soil (fig. 3).

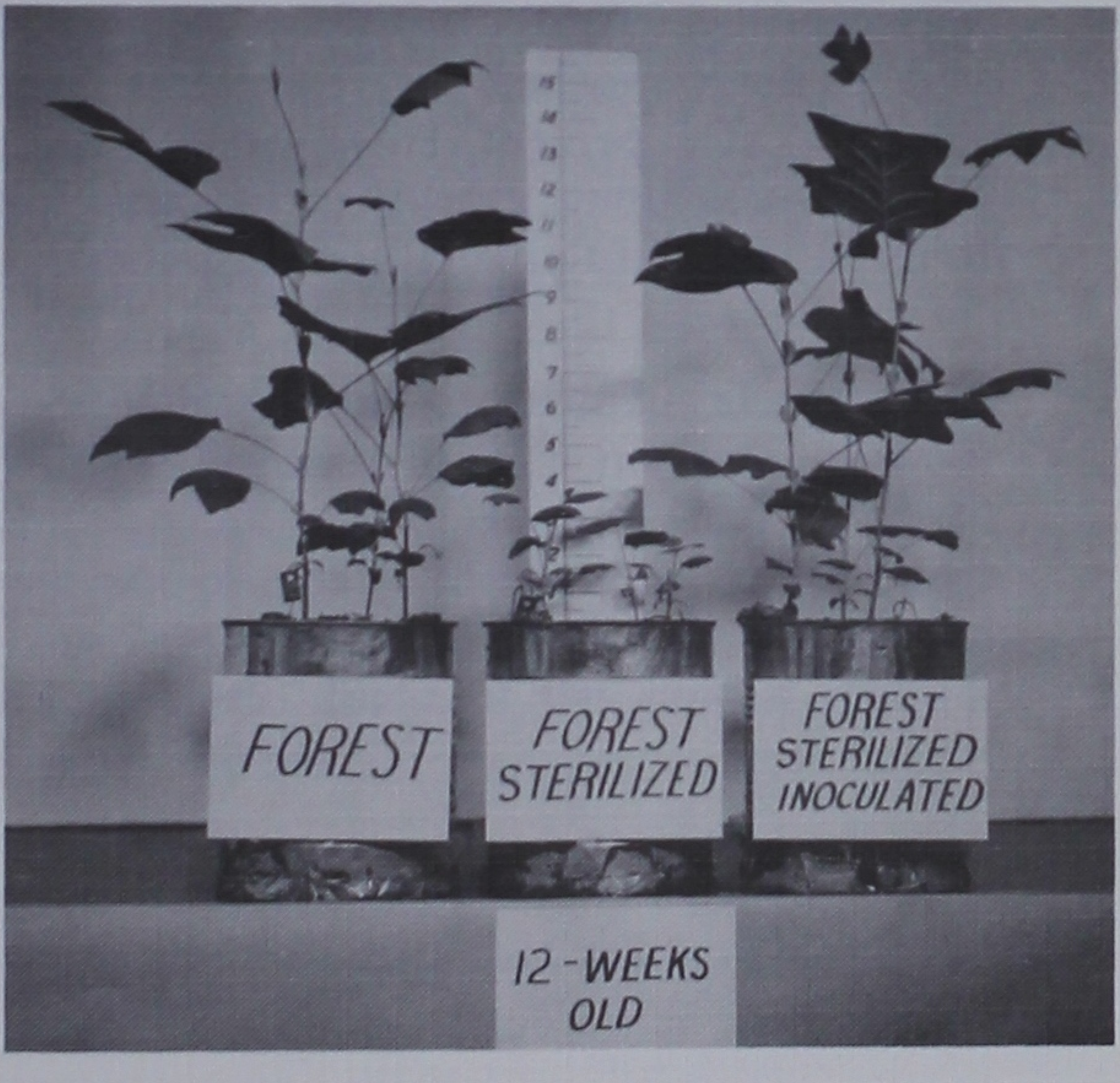

FIGURE 3. — Seedlings grown in sterilized soil were small and chlorotic, but the addition of macerated yellow-poplar root inoculum greatly increased growth.

Microscopic examination of sections of the roots in three different trials showed that seedlings in sterilized soil were nonmycorrhizal, while seedlings in UN sterilized and sterilized-inoculated soil were infected with endotrophic mycorrhizal fungi. Yellow-poplar is known to be naturally infected with a fungus that forms endotrophic mycorrhizae. The well-known ectotrophic mycorrhizal fungi found on pines and other species form a fungal sheath and mycelial strands penetrate between cells of the roots. In contrast the lesser known endotrophic mycorrhizal fungi do not form a fungal sheath but grow into root cells.

Although seedling growth in old-field soil was poor, the seedlings were infected by mycorrhizal fungi. However, the hyphae within the cells of these old-field seedling roots appeared to be disintegrating. Seedlings from sieved containers were only weakly infected with mycorrhizal fungi.

Mycorrhizae were prevalent in seedlings grown in forest soil containers fertilized with 6-24-12 and in unfertilized containers. In unsterilized forest soil, fertilized seedlings at 12 weeks weighed 9.7 grams compared with 7.7 grams for unfertilized seedlings. This difference was significant at the 1 percent level. In sterilized containers the fertilized seedlings weighed 4.2 grams and unfertilized seedlings weighed only 1.6 grams (fig. 4); the difference was significant at 1 percent. However, the addition of nitrogen failed to increase seedling growth in old-field soil: seedlings in neither sterilized nor unsterilized soil responded to ammonium nitrate.

FIGURE 4. — Addition of 600 pounds per acre of 6-24-12 fertilizer to sterilized forest soil nearly tripled growth.

Discussion

Study results show that old-field sites are improving for hardwoods under planted and natural tree cover. Auten[3] found that old-field soil structure, measured in terms of infiltration rate, improves under sassafras, black locust, and pines. Also it is commonly observed that the better hardwood species are invading both planted and volunteer stands on old fields. Attempts to plant these same species in open old fields have generally failed.

The current study provides a way to evaluate site changes in terms of seedling growth in contrast to such measures as infiltration rate, pore volume, organic matter content, and so forth. Since the study sites were not originally identical, absolute cause and effect comparisons are not justifiable. But the seedling-size differences among sites leave little doubt of the general relation between cover and soil improvement.

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[3]Auten, John T. Relative influence of sassafras, black locust, and pines upon old-field soils. Jour. Forestry 43: 441-446. 1945.

The soil under shortleaf pine and black locust plantations and fully stocked volunteer stands is improving for yellow-poplar seedling growth. However, partly stocked volunteer stands have not greatly influenced soil conditions. Partly stocked old fields are common throughout the Central States and most will become fully stocked with time. If the rate of this natural invasion is slow, then field openings should be planted to get the land back into full productivity as soon as possible.

Soil structure appears to be a main difference between forest and old-field soils. Where macro-soil structure was destroyed the organic-matter content and abundance of mineral elements were essentially the same as in undisturbed soil. Yet sieving greatly reduced growth. While sieving probably did not destroy the smaller soil aggregates, pore volume was reduced. Forest soils have a complex, porous structure but old-field soils are typically more dense. In soils that have poor structure and poor aeration yellow-poplar seedlings apparently cannot absorb sufficient nutrients and/or water and oxygen for good growth. In future work to improve old-field sites for growing hardwoods the importance of soil structure should be considered.

This series of studies has clearly demonstrated that endotrophic mycorrhizae are essential to the normal development of yellow-poplar. The occurrence of these associations has been known for some time but their effect on host plants has not been established. Research on ectotrophic mycorrhizae has shown that the host plant absorbs more nutrients when mycorrhizae are present on the roots. This relation remains to be proved for endotrophic mycorrhizae. Mosse[4] has shown in England that rooted apple cuttings infected with an endotroph were usually larger than uninfected cuttings. Baylis[5] of New Zealand demonstrated that *Griselinia* (Cornaceae) seedlings infected with endotrophic mycorrhizal fungi were larger after 1 to 2 years than uninfected seedlings grown in sterilized soil. The present study with yellow-poplar

[4]Mosse, B. Growth and chemical composition of mycorrhizal and nonmycorrhizal apples. Nature 179: 922-924. 1957.

[5]Baylis, G. T. S. Effect of vesicular arbuscular mycorrhizas on growth of *Griselinia littoralis* (Cornaceae). New Phytologist 58: 274-280. 1959.

may be the first clear-cut demonstration of the effects of endotrophic mycorrhizae on the growth of a commercially important forest tree.

Seedlings grown in old-field soil were found to be infected with mycorrhizal fungi so lack of mycorrhizae is likely not a problem in planting hardwoods on old-field sites. However, the hyphae in roots of seedlings from old-field soil appeared to be in poor condition. Probably old fields are poor sites for mycorrhizal development or mycorrhizae develop poorly on slow-growing plants.

Another interesting feature in mycorrhizae development was a time lag in the growth response of seedlings in soil that was sterilized and inoculated. At 7 weeks there was no difference in height between seedlings in sterilized containers and sterilized-inoculated containers. During the final 5 weeks of growth, seedlings in the inoculated soil grew fast enough to catch up with seedlings in unsterilized soil. Either the fungus needs some time to make effective contact with the roots or the mycorrhizal fungi must reach a certain level of abundance to become effective.

The reasons for poor growth of seedlings without mycorrhizae are still unknown. Like ectotrophic mycorrhizae, the role of the endotroph may be that of increased nutrient absorption. This premise remains to be proved experimentally. The fact that seedlings in containers of fertilized soil with mycorrhizal fungi grew faster than fertilized seedlings without mycorrhizae supports the theory of nutrient absorption by seedlings through mycorrhizae. It has been established that the abundance of ectotrophic mycorrhizal fungi varies inversely with the nutrient level of the soil in which a plant is grown. In this study the amount of fertilizer used did not affect mycorrhizal formation. However, the question of abundance of endotrophs and fertility remains open. With the widespread use of soil fumigants and liberal application of fertilizers in forest nurseries, the findings of these studies may have some practical implications.

Summary

Old-field soil conditions under planted black locust and shortleaf pine and under a fully stocked volunteer hardwood stand have improved for seedling growth over a 23-year period. Soil structure was judged to be the most important difference between old-field and adjacent forest sites. For normal growth and development yellow-poplar seedlings must be infected with fungi that form endotrophic mycorrhizae. Seedlings grown in old-field soil are mycorrhizal but the fungi appear to be in poor condition. Preliminary trials with the addition of a complete fertilizer support the theory that the role of endotrophic mycorrhizae is in seedling nutrition.

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